

WHAT IS CLAIMED IS:

1. A gene which encodes reverse transcriptase having DNA polymerase activity and substantially no RNase H activity.

2. The gene of claim 1, wherein said gene is derived from an organism selected from the group consisting of Moloney murine leukemia virus (M-MLV), human T-cell leukemia virus type I (HTLV-I), bovine leukemia virus (BLV), Rous sarcoma virus (RSV), human immunodeficiency virus (HIV), yeast, Neurospora, Drosophila, primates and rodents.

3. The gene of claim 1, wherein said microorganism is M-MLV, comprising the following DNA sequence:

ATG ACC CTA AAT ATA GAA GAT GAG CAT CGG CTA CAT GAG ACC TCA AAA GAG CCA GAT GTT 1078
TCT CTA GGG TOC ACA TGG CTG TCT GAT TTT OCT CAG GOC TGG GCG GAA ACC GGG GGC ATG 1138
GGA CTG GCA GTT GGC CAA GCT OCT CTG ATC ATA OCT CTG AAA GCA ACC TCT ACC CCC GIG 1198
TOC ATA AAA CAA TAC OOC ATG TCA CAA GAA GOC AGA CTG GGG ATC AAG OOC CAC ATA CAG 1258
AGA CTG TTG GAC CAG GGA ATA CTG GTA OOC TGC CAG TOC OOC TGG AAC ACG OOC CTG CTA 1318
OOC GTT AAG AAA CCA GGG ACT AAT GAT TAT AGG OCT GTC CAG GAT CTG AGA GAA GTC AAC 1378
AAG CGG GTG GAA GAC ATC CAC OOC AOC GTG OOC AAC OCT TAC AAC CTC TTG AGC GGG CTC 1438
CCA CGG TOC CAC CAG TGG TAC ACT GIG CTT GAT TTA AAG GAT GOC TTT TTC TGC CTG AGA 1498
CTC CAC OOC AOC AGT CAG OCT CTC TTC GOC TTT GAG TGG AGA GAT CCA GAG ATG GGA ATC 1558
TCA GGA CAA TTG AOC TGG AOC AGA CTC CCA CAG GGT TTC AAA AAC AGT OOC ACC CTG TTT 1618
GAT GAG GCA CTG CAC AGA GAC CTA GCA GAC TTC CGG ATC CAG CAC CCA GAC TTG ATC CTG 1678
CTA CAG TAC GTG GAT GAC TTA CTG CTG GOC GOC ACT TCT GAG CTA GAC TGC CAA CAA GGT 1738
ACT CGG GOC CTG TTA CAA AOC CTA GGG AAC CTC GGG TAT CGG GOC TCG GOC AAG AAA GOC 1798
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1858
 CAA ATT TGC CAG AAA CAG GTC AAG TAT CTG GGG TAT CTT CTA AAA GAG GGT CAG AGA TGG
 1918
 CTG ACT GAG GGC ACA AAA GAG ACT GTG ATG GGG CAG OCT ACT CCG AAG ACC OCT CGA CAA
 1978
 CTA AGG GAG TTC CTA GGG ACG GCA GGC TTC TGT GGC CTC TGG ATC OCT GGG TTT GCA GAA
 2038
 ATG GCA GGC CCC TTG TAC OCT CTC ACC AAA ACG GGG ACT CTG TTT AAT TGG GGC CCA GAC
 2098
 CAA CAA AAG GGC TAT CAA GAA ATC AAG CAA GCT CTT CTA ACT GGC CCA GGC CTG GGG TTG
 2158
 CCA GAT TTG ACT AAG CCC TTT GAA CTC TTT GTC GAC GAG AAG CAG GGC TAC GGC AAA GGT
 2218
 GTC CTA ACG CAA AAA CTG GGA OCT TGG CGT CCG CCG GTG GGC TAC CTG TOC AAA AAG CTA
 2278
 GAC CCA GTA GCA GCT GGG TGG CCC OCT TGC CTA CCG ATG GTA GCA GGC ATT GGC GTA CTG
 2338
 ACA AAG GAT GCA GGC AAG CTA ACC ATG GGA CAG CCA CTA GTC ATT CTG GGC CCC CAT CCA
 2398
 GTA GAG GCA CTA GTC AAA CAA CCC CCG GAC CCG TGG CTT TOC AAC GGC CCG ATG ACT CAC
 2458
 TAT CAG GGC TTG CTT TTG GAC ACG GAC CCG GTC CAG TTC GGA CCG GTG GTA GGC CTG AAC
 CCG GCT ACG CTG CTC CCA CTG OCT GAG GAA GGG CTG CAA CAC AAC TGC CTT GAT

or the degenerate variants thereof.

4. The gene of claim 1, wherein said micro-organism is M-MLV, comprising the following DNA sequence:

1078
 ATG ACC CTA AAT ATA GAA GAT GAG CAT CCG CTA CAT GAG ACC TCA AAA GAG CCA GAT GTT
 1138
 TCT CTA GGG TOC ACA TGG CTG TCT GAT TTT OCT CAG GGC TGG GCG GAA ACC GGG GGC ATG
 1198
 GGA CTG GCA GTT CCG CAA GCT OCT CTG ATC ATA OCT CTG AAA GCA ACC TCT ACC CCC GTG
 1258
 TOC ATA AAA CAA TAC CCC ATG TCA CAA GAA GGC AGA CTG GGG ATC AAG CCC CAC ATA CAG
 1318
 AGA CTG TTG GAC CAG GGA ATA CTG GTA CCC TGC CAG TOC CCC TGG AAC ACG CCC CTG CTA
 1378
 CCC GTT AAG AAA CCA GGG ACT AAT GAT TAT AGG OCT GTC CAG GAT CTG AGA GAA GTC AAC
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1438
AAG CGG GIG GAA GAC ATC CAC CCC ACC GIG CCC AAC OCT TAC AAC CTC TTG AGC GGG CTC

1498
CCA CCG TOC CAC CAG TGG TAC ACT GIG CIT GAT TTA AAG GAT GOC TTT TTC TGC CTG AGA

1558
CTC CAC CCC ACC AGT CAG OCT CTC TTC GOC TTT GAG TGG AGA GAT CCA GAG ATG GGA ATC

1618
TCA GGA CAA TTG ACC TGG ACC AGA CTC CCA CAG GGT TTC AAA AAC AGT CCC ACC CTG TTT

1678
GAT GAG GCA CTG CAC AGA GAC CTA GCA GAC TTC CGG ATC CAG CAC CCA GAC TTG ATC CTG

1738
CTA CAG TAC GIG GAT GAC TTA CTG CTG GOC GOC ACT TCT GAG CTA GAC TGC CAA CAA GGT

1798
ACT CGG GOC CTG TTA CAA ACC CTA GGG AAC CTC GGG TAT CGG GOC TOG GOC AAG AAA GOC

1858
CAA ATT TGC CAG AAA CAG GTC AAG TAT CTG GGG TAT CTT CTA AAA GAG GGT CAG AGA TGG

1918
CTG ACT GAG GOC AGA AAA GAG ACT GIG ATG GGG CAG OCT ACT CCG AAG ACC OCT CGA CAA

1978
CTA AGG GAG TTC CTA GGG ACG GCA GGC TTC TGT CGC CTC TGG ATC OCT GGG TTT GCA GAA

2038
ATG GCA GOC CCC TTG TAC OCT CTC ACC AAA ACG GGG ACT CTG TTT AAT TGG GGC CCA GAC

2098
CAA CAA AAG GOC TAT CAA GAA ATC AAG CAA GCT CTT CTA ACT GOC CCA GOC CTG GGG TTG

2158
CCA GAT TTG ACT AAG CCC TTT GAA CTC TTT GTC GAC GAG AAG CAG GGC TAC GOC AAA GGT

2218
GTC CTA ACG CAA AAA CTG GGA OCT TGG CGT CGG CCG GIG GOC TAC CTG TOC AAA AAG CTA

2278
GAC CCA GTA GCA GCT GGG TGG CCC OCT TGC CTA CGG ATG GTA GCA GOC ATT GOC GTA CTG

2338
ACA AAG GAT GCA GGC AAG CTA ACC ATG GGA CAG CCA CTA GTC ATT CTG GOC CCC CAT GCA

2398
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GTA GAG GCA CTA GTC AAA CAA CCC CCC GAC CGC TGG CTT TOC AAC GOC CGG ATG ACT CAC
2458
TAT CAG GOC TTG CTT TTG GAC ACG GAC CGG GTC CAG TTC GGA OCG GIG GTA GOC CTG AAC
2518
CCG GCT ACG CTG CTC CCA CTG CCT GAG GAA GGG CTG CAA CAC AAC TGC CTT GAT AAT TOC
2530
CGC TTA ATT AAT

or the degenerate variants thereof.

5. A gene which encodes a fusion protein which comprises reverse transcriptase having DNA polymerase activity and substantially no RNase H activity and a second protein comprising a hydrophobic leader peptide or a stabilizing peptide.

6. A vector containing the gene of claim 1 or 5.

7. The vector of claim 6 designated pRTdEcoRV-C which has been deposited at the American Type Culture Collection, Rockville Maryland under terms of the Budapest Treaty and given accession number 67555.

8. A host transformed with the vector of claim 6.

9. A polypeptide having an amino acid sequence encoded by the cloned gene of claim 1 or 5.

10. The polypeptide of claim 9 comprising the following amino acid sequence:

MET Thr Leu Asn Ile Glu Asp Glu His Arg Leu His Glu Thr Ser Lys Glu Pro Asp Val
Ser Leu Gly Ser Thr Trp Leu Ser Asp Phe Pro Gln Ala Trp Ala Glu Thr Gly Gly MET
Gly Leu Ala Val Arg Gln Ala Pro Leu Ile Ile Pro Leu Lys Ala Thr Ser Thr Pro Val
Ser Ile Lys Gln Tyr Pro MET ser Gln Glu Ala Arg Leu Gly Ile Lys Pro His Ile Gln
Arg Leu Leu Asp Gln Gly Ile Leu Val Pro Cys Gln Ser Pro Trp Asn Thr Pro Leu Leu
Pro Val Lys Lys Pro Gly Thr Asn Asp Tyr Arg Pro Val Gln Asp Leu Arg Glu Val Asn
Lys Arg Val Glu Asp Ile His Pro Thr Val Pro Asn Pro Tyr Asn Leu Leu Ser Gly Leu
Pro Pro Ser His Gln Trp Tyr Thr Val Leu Asp Leu Lys Asp Ala Phe Phe Cys Leu Arg
Leu His Pro Thr Ser Gln Pro Leu Phe Ala Phe Glu Trp Arg Asp Pro Glu MET Gly Ile
Ser Gly Gln Leu Thr Trp Thr Arg Leu Pro Gln Gly Phe Lys Asn Ser Pro Thr Leu Phe
Asp Glu Ala Leu His Arg Asp Leu Ala Asp Phe Arg Ile Gln His Pro Asp Leu Ile Leu
Leu Gln Tyr Val Asp Asp Leu Leu Leu Ala Ala Thr Ser Glu Leu Asp Cys Gln Gln Gly
Thr Arg Ala Leu Leu Gln Thr Leu Gly Asn Leu Gly Tyr Arg Ala Ser Ala Lys Lys Ala
Gln Ile Cys Gln Lys Gln Val Lys Tyr Leu Gly Tyr Leu Leu Lys Glu Gly Gln Arg Trp
Leu Thr Glu Ala Arg Lys Glu Thr Val MET Gly Gln Pro Thr Pro Lys Thr Pro Arg Gln
Leu Arg Glu Phe Leu Gly Thr Ala Gly Phe Cys Arg Leu Trp Ile Pro Gly Phe Ala Glu
MET Ala Ala Pro Leu Tyr Pro Leu Thr Lys Thr Gly Thr Leu Phe Asn Trp Gly Pro Asp
Gln Gln Lys Ala Tyr Gln Glu Ile Lys Gln Ala Leu Leu Thr Ala Pro Ala Leu Gly Leu
Pro Asp Leu Thr Lys Pro Phe Glu Leu Phe Val Asp Glu Lys Gln Gly Tyr Ala Lys Gly
Val Leu Thr Gln Lys Leu Gly Pro Trp Arg Arg Pro Val Ala Tyr Leu Ser Lys Lys Leu
Asp Pro Val Ala Ala Gly Trp Pro Pro Cys Leu Arg MET Val Ala Ala Ile Ala Val Leu
Thr Lys Asp Ala Gly Lys Leu Thr MET Gly Gln Pro Leu Val Ile Leu Ala Pro His Ala
Val Glu Ala Leu Val Lys Gln Pro Pro Asp Arg Trp Leu Ser Asn Ala Arg MET Thr His

FOOTNOTES

Tyr Gln Ala Leu Leu Leu Asp Thr Asp Arg Val Gln Phe Gly Pro Val Val Ala Leu Asn
Pro Ala Thr Leu Leu Pro Leu Pro Glu Glu Gly Leu Gln His Asn Cys Leu Asp.

11. The polypeptide of claim 9 comprising the following amino acid sequence:

MET Thr Leu Asn Ile Glu Asp Glu His Arg Leu His Glu Thr Ser Lys Glu Pro Asp Val
Ser Leu Gly Ser Thr Trp Leu Ser Asp Phe Pro Gln Ala Trp Ala Glu Thr Gly Gly MET
Gly Leu Ala Val Arg Gln Ala Pro Leu Ile Ile Pro Leu Lys Ala Thr Ser Thr Pro Val
Ser Ile Lys Gln Tyr Pro MET ser Gln Glu Ala Arg Leu Gly Ile Lys Pro His Ile Gln
Arg Leu Leu Asp Gln Gly Ile Leu Val Pro Cys Gln Ser Pro Trp Asn Thr Pro Leu Leu
Pro Val Lys Lys Pro Gly Thr Asn Asp Tyr Arg Pro Val Gln Asp Leu Arg Glu Val Asn
Lys Arg Val Glu Asp Ile His Pro Thr Val Pro Asn Pro Tyr Asn Leu Leu Ser Gly Leu
Pro Pro Ser His Gln Trp Tyr Thr Val Leu Asp Leu Lys Asp Ala Phe Phe Cys Leu Arg
Leu His Pro Thr Ser Gln Pro Leu Phe Ala Phe Glu Trp Arg Asp Pro Glu MET Gly Ile
Ser Gly Gln Leu Thr Trp Thr Arg Leu Pro Gln Gly Phe Lys Asn Ser Pro Thr Leu Phe
Asp Glu Ala Leu His Arg Asp Leu Ala Asp Phe Arg Ile Gln His Pro Asp Leu Ile Leu
Leu Gln Tyr Val Asp Asp Leu Leu Leu Ala Ala Thr Ser Glu Leu Asp Cys Gln Gln Gly
Thr Arg Ala Leu Leu Gln Thr Leu Gly Asn Leu Gly Tyr Arg Ala Ser Ala Lys Lys Ala
Gln Ile Cys Gln Lys Gln Val Lys Tyr Leu Gly Tyr Leu Leu Lys Glu Gly Gln Arg Trp
Leu Thr Glu Ala Arg Lys Glu Thr Val MET Gly Gln Pro Thr Pro Lys Thr Pro Arg Gln
Leu Arg Glu Phe Leu Gly Thr Ala Gly Phe Cys Arg Leu Trp Ile Pro Gly Phe Ala Glu
MET Ala Ala Pro Leu Tyr Pro Leu Thr Lys Thr Gly Thr Leu Phe Asn Trp Gly Pro Asp
Gln Gln Lys Ala Tyr Gln Glu Ile Lys Gln Ala Leu Leu Thr Ala Pro Ala Leu Gly Leu
Pro Asp Leu Thr Lys Pro Phe Glu Leu Phe Val Asp Glu Lys Gln Gly Tyr Ala Lys Gly
Val Leu Thr Gln Lys Leu Gly Pro Trp Arg Arg Pro Val Ala Tyr Leu Ser Lys Lys Leu

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Asp Pro Val Ala Ala Gly Trp Pro Pro Cys Leu Arg MET Val Ala Ala Ile Ala Val Leu
Thr Lys Asp Ala Gly Lys Leu Thr MET Gly Gln Pro Leu Val Ile Leu Ala Pro His Ala
Val Glu Ala Leu Val Lys Gln Pro Pro Asp Arg Trp Leu Ser Asn Ala Arg MET Thr His
Tyr Gln Ala Leu Leu Leu Asp Thr Asp Arg Val Gln Phe Gly Pro Val Val Ala Leu Asn
Pro Ala Thr Leu Leu Pro Leu Pro Glu Glu Gly Leu Gln His Asn Cys Leu Asp Asn Ser
Arg Leu Ile Asn.

12. A method of producing reverse transcriptase having DNA polymerase activity and substantially no RNase H activity comprising culturing the transformed host of claim 8 under conditions which produce reverse transcriptase, and isolating the reverse transcriptase so produced.

13. A method of preparing cDNA from mRNA, comprising

(a) contacting mRNA with an oligo(dT) primer or other complementary primer to form a hybrid, and

(b) contacting said hybrid formed in step (a) with reverse transcriptase, having DNA polymerase and substantially no RNase activity, and the nucleoside triphosphates to give a cDNA-RNA hybrid.

14. The method of claim 13, further comprising treating the cDNA-RNA with alkali or RNase H to selectively hydrolyze said RNA to give a cDNA.

15. The method of claim 13, further comprising treating said cDNA with DNA polymerase to give second-strand cDNA.

16. A kit for the preparation of cDNA from mRNA, comprising a carrier means being compartmentalized to receive in close confinement therein, one or more containers wherein

(a) a first container contains reverse transcriptase having DNA polymerase activity and substantially no Rnase H activity;

(b) a second container contains the nucleoside triphosphates, and

(c) a third container contains oligo(dT) primer.

17. The kit of claim 16, further comprising:

(d) a fourth container containing control RNA.

18. The kit of claim 16, wherein said second container further contains a buffer.

19. The kit of claim 16, wherein said reverse transcriptase is present at a concentration of 200 $\mu\text{g}/\mu\text{l}$ to 400 $\mu\text{g}/\mu\text{l}$.

20. The kit of claim 16, wherein said oligo (dT) primer is present at a concentration of 5 $\mu\text{g}/\text{ml}$ to 20 $\mu\text{g}/\text{ml}$.

21. The kit of claim 18, wherein said buffer comprises Tris-HCl (pH 7.5 to 8.3), KCl, MgCl_2 , and dithiothreitol.

22. The kit of claim 16, wherein said nucleoside triphosphates are present at a concentration of 0.5 mM to 2 mM.

23. The kit of claim 17, wherein said control RNA is present at a concentration of 10 μ g/ml to 20 μ g/ml.

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